Effect of Poly (Toluene Sulphonic Acid) in Enhancing Durability of Poly (Pyrrole)/Poly (N-Methylpyrrole)/GOx Composite Glucose Biosensor

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Abstract-**The effect of poly (tolune sulphonic acid) in enhancing durability of (poly) pyrrole/ (poly) N-methyl pyrrole/ glucose oxidase sensor has been reported in the present investigation. The (poly) pyrrole/ (poly) N-methyl pyrrole composite matrix was synthesized by galvanostatic technique. The poly (toluene sulphonic acid) was added as dopant to enhance susceptibility of the synthesized matrix towards humidity. The polypyrrole – poly (n-methylpyrrole) composite films were subjected to spectral and morphological characterizations. Excellent morphology, suitable for immobilization of glucose–oxidase, was achieved with optimized process parameters. The glucose–oxidase was immobilized on the composite matrix by cross linking via glutaraldehyde. Comparative study was carried out to investigate functionality of the sensor in phosphate and acetate buffer atmosphere. The biosensor exhibited an excellent linear response for a wide range of glucose concentration from 0mM to 10mM in both the buffers. However the sensitivity of the developed biosensor in presence of phosphate buffer (0.41 µA/mM) was found to be higher. The durability of the sensor was found to be excellent (47 days) in presence of poly (toluene sulphonic acid) in phosphate buffer.**

Keywords-Glucose biosensor; Polypyrrole; Poly (N-methyl pyrrole); Composite; Electropolymerization; Poly (Toluene sulphonic acid)

I. INTRODUCTION

Organic Conducting Polymers (OCPs) are well known to be used as enzyme immobilization matrix for development of biosensors [1]–[6]. Extensive study has revealed that a varied spectrum of functionalities can be incorporated in OCP matrix during or post polymerization condition [7]–[10]. However, an efficient immobilization invariably depends on the macro properties of the matrix (vie surface morphology, adhesively etc.) that need to be tuned by optimizing polymerization conditions (viz type of dopant monomer: dopant conc., deposition current etc.) for reliable and reproducible transduction mechanisms [11] – [14].

The estimation of glucose concentration in blood constitutes an important and high- in demand sector in medical diagnostics. The present paradigm of glucose sensing is primarily based on redox properties of Glucose Oxidase (GODx) and several biosensors have been reported utilizing the mechanism [15]-[17]. OCPs are attractive for immobilization of biocomponents, which is fundamentally due to the fact that transduction in OCPs takes place at room temperature which is a prerequisite for proper functioning of biomolecules. Moreover, the conductivity level of OCP films can be controlled to suit the specific requirements of enzymatic activity. However the foremost problem that is encountered by any OPC-based sensors is the degradation of the same under even normal atmospheric condition. The most popular OCPs viz. Polyaniline, Polypyrrole etc. are reported to suffer such degradation [18], [19]. Among various factors, the presence of oxygen and humidity plays the most prominent role in destabilizing the performance of OCP sensors.

In pursuit towards a way out of this problem we have employed a composite Poly (pyrrole) [P (py)]/Poly (N-methyl pyrrole) [P/NMP] matrix for glucose sensing application [20]. In that approach, less susceptibility of P (NMP) and oxygen and humidity was an added advantage over the well-known biocompatibility, high conductivity [21], and ease of synthesis of P (Py).

The present investigation is a continuation of our previous studies. Here we report a glucose sensor with P (Py)/P (NMP) composite matrix synthesized with Ploy (Toluene Sulphonic Acid) (pTS) as a dopant. The choice of dopant was supported by the fact that presence of pTS in OCP atmosphere is reported to cause a reduction in moisture content [22]. Moreover, one of our composite P (Py)/P (NMP) film synthesized with pTS as a dopant exhibit excellent electrical and structural properties are highly suitable for enzyme entrapment.

II. EXPERIMENTAL PART

A. Preparation of P(Py)/ P(NMP)/ pTS Film

Chronopotentiometric technique was employed for the synthesis of P (Py)/P (NMP)/pTS composite film. The electrolyte solution (20ml) consisted of 0.1M Py, 0.1M NMP and 0.1M pTS in double de-ionized water. The monomers Py (98.1%) and NMP (98%) were obtained from Alfa Aesar and Acros Organics respectively. Dopant pTS was procured from Rankem (India). The monomers were double distilled prior synthesis. The synthesis was carried out in a conventional three electrode cell with planar Platinum foil as working and counter electrode and Ag/AgCl as reference electrode. Optimized current density (1mA/cm^2), deposition time (5 min) and pH (3.0) were employed for efficient surface morphology. The synthesis was carried out under ambient conditions. The synthesized films (on platinum substrate) were rinsed with double deionised water to wash out loosely-bounded particles and dried in normal conditions. The synthesis and electrochemical characterization was carried away with CHI 660C electrochemical workstation. The formation of P (Py)/P (NMP) composite was confirmed by FTIR spectral study

using Testscan Shimadzu FTIR- 800 series UV –Visible spectrophotometer in region between 400 and 4000 cm^{-1}. Scanning Electron Micrographs were recorded at different magnifications using JEOL JSM-630, an analytical scanning electron microscope.

B. Immobilization of GOx on P(Py)/ P(NMP)/ pTs Film

The stock solutions of Glucose oxidaxe (GOx) (Aldrich) (1mg/ml) were prepared in 0.1M phosphate buffer and acetate buffers respectively. The solutions were kept under continuous and moderate stirring for 24 h before use. GOx was finally immobilized by cross linking via (0.1%) glutaraldehyde (Loba Chemie, India) on P (Py)/ P (NMP)/ pTS films. A 30 min. incubation time was allotted followed by repeated phosphate and/or acetate buffer wash to ensure that the enzyme is not leached out of the film. Optimized concentrations of GOx and glutaraldehyde were employed for efficient enzyme loading and retention [23].

C. Amperometric Determination of Glucose

The stock solution of D-Glucose was prepared in phosphate (0.1 M) and acetate (0.1M) buffers respectively before 24 h of testing and stored at 4^0C. Amperometric response of the biosensor was studied, for gradually increasing concentrations (0-50M) of glucose, using CHI 660C electrochemical workstation. P (Py)/P (NMP)/pTS electrode was maintained at +700 mV version Ag/Agcl reference electrode in phosphate and acetate buffer solution respectively to yield a stable background current [24].

III. RESULTS AND DISCUSSION

A. Sensing Mechanism

In GOx based glucose sensor, the added glucose solution is oxidised redox action of immobilized GOx in presence of dissolved O_2. The following reaction depicts the phenomenon

The generated H_2O_2 is further oxidized at the polymer layer and results in an anodic current according to the following relation-

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconic acid} + H_2O_2 \quad (1)$$

$$H_2O_2 \xrightarrow{\text{PPY/NMP}} O_2 + 2H^+ + 2e^- \quad (2)$$

The conversion of glucose to gluconic acid results in transfer of two protons and two electrons from the substrate to enzyme [25]. The conducting polymer facilitates the transfer of electrons from redox- cofactor to the sensing electrode.

B. Electrochemical Synthesis of P(Py)/P(NMP)/pTS Composite Film

P (Py)/P (NMP)/pTS composite films were synthesized galvanostatically. Optimized process parameters were employed for synthesis. A low polymerization potential was recorded in chronopotentiogram that ensures higher conductivity and uniform surface morphology. Synthesized composite films had uniform morphology and good adhesivity.

C. FTIR Spectra of P(Py)/P(NMP)/pTS Composite Film

The Principal absorption band observed in the FTIR spectrum of the P (Py)/P (NMP)/pTS composite film (Figure not included). The broad peak at 3695 cm^{-1} corresponds to N-H stretching. The absorption band observed at 2779 cm^{-1} is due to C-H vibrations, and the C = O stretching is observed at 1741 cm^{-1}. The band observed at 1452 cm^{-1} is due to C=C stretching. The peak at 2962 cm^{-1} is attributed to the CH_3 stretching. The sharp absorption band observed at 1259 cm^{-1} is due to the ring stretch of n-methyl pyrrole. The FTIR spectral result confirms the formation of poly pyrrole P(Py) and poly n-methyl pyrrole P(NMP).

D. SEM Study of P(Py)/P(NMP)/pTS Film

The SEM image (Fig.1) for pristine P (Py)/P (NMP)/pTS composite film reveals uniform and granular surface morphology. Such morphology is highly suitable for entrapment of enzyme thus rendering higher stability to the biosensor [26].

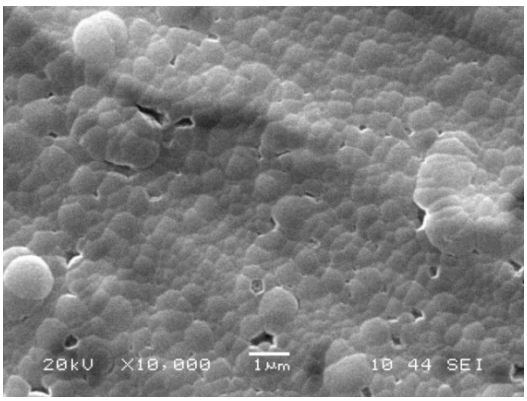

Fig. 1 SEM image of P(Py)/P(NMP)/pTS composite film with optimized process parameters

E. Sensing Response of P(Py)/P(NMP)/pTS/GOx Electrode

The sensitivity of the glucose biosensor is dependent of the GOx entrapped in the electrodeposited polypyrrole film. As it is described earlier, the response of biosensor is proportional to the concentration of H_2O_2 catalytically produced by the GOx on the anode. Thus, the sensing current can determine the relative activity of immobilized GOx.

The P (Py)/P (NMP)/pTS/GOx biosensor responded well in both acetate and phosphate buffer. However, better transduction was recorded for phosphate buffer. The amperometric responses (with electrodes kept at +700mV versus Ag/AgCl reference electrode) of the sensing electrodes in presence of phosphate and acetate buffer are shown in Fig. 2 and Fig. 3 respectively.

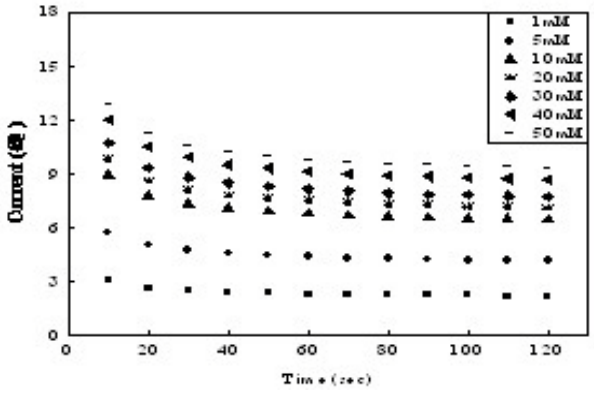

Fig. 2 Current-time relationship(sensing response) of P(Py)/P(NMP)/pTS/GOx electrode in 0.1M phosphate buffer (pH7.4) for different glucose concentrations of 0-50mM: a) 0mM ; b) 1mM ; c) 2mM ; d) 3mM ; e) 4mM ; f) 5mM ; g) 6mM ; h) 7mM ; i) 8mM ; j) 9mM ; k) 10mM ; l) 20mM ; m) 30mM; n) 40mM ; o) 50mM .

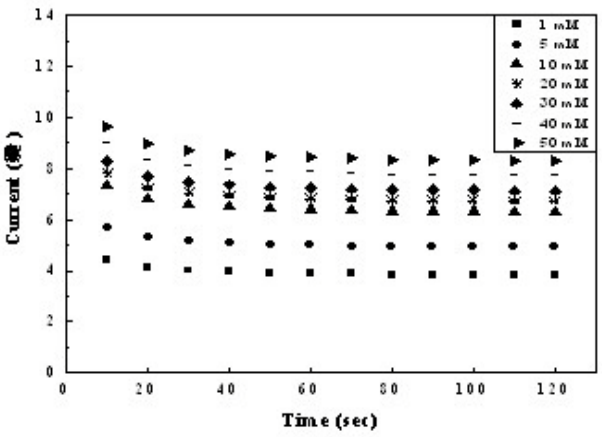

Fig. 3 Current-time relationship(sensing response) of P(Py)/P(NMP)/pTS/GOx electrode in 0.1M acetate buffer (pH7.4) for different glucose concentrations of 0-50mM: a) 0mM ; b) 1mM ; c) 2mM ; d) 3mM ; e) 4mM ; f) 5mM ; g) 6mM ; h) 7mM ; i) 8mM ; j) 9mM ; k) 10mM ; l) 20mM ; m) 30mM; n) 40mM ; o) 50mM .

The response current was found to reach a steady state easily. The relationship between response current and glucose concentration (0-10mM) in 0.1M phosphate buffer (pH 7.4) is shown in Fig.4 (the linear regression equation is $y = 0.499x + 1.548$ and the linear regression coefficient is $R^2 = 0.983$). The inset indicates a linear response from 0mM to 50mM of glucose concentration (The linear regression equation is $y = 0.141x + 3.314$and the linear regression coefficient is $R^2 = 0.797$).

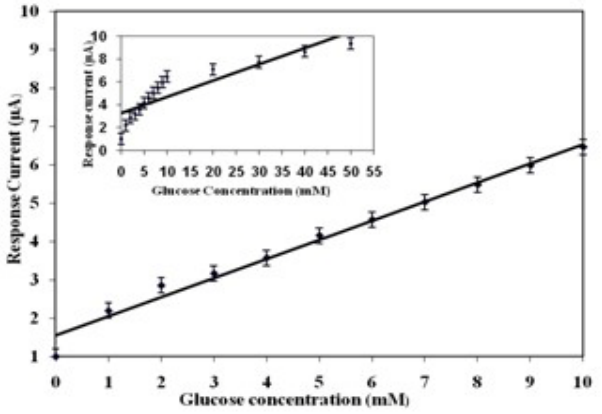

Fig. 4 The relationship between response current and glucose concentration for P(Py)/P(NMP)/pTS/GOx electrode in 0.1M phosphate buffer (pH7.4). The inset indicates the response from 10mM to 50mM.

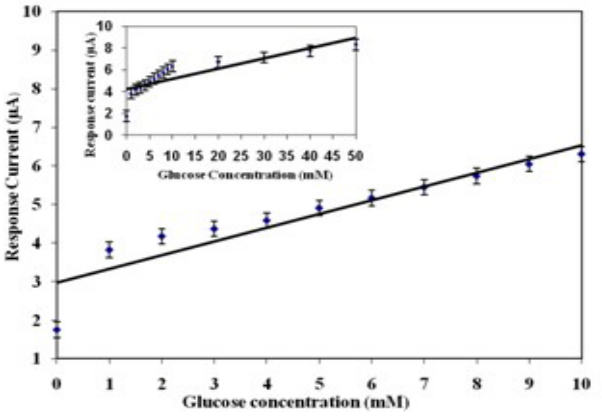

Fig. 5 The relationship between response current and glucose concentration for P(Py)/P(NMP)/pTS/GOx electrode in 0.1M acetate buffer (pH7.4). The inset indicates the response from 10mM to 50mM.

Similarly the linear relationship between response current and glucose concentration (0-10M) in 0.1M acetate buffer (pH 7.4) is shown in Fig. 5 (the linear regression equation is $y= 0.445x+2.954$ and the linear regression coefficient is $R^2= 0.911$). The inset indicates a linear response from 0mM to 50mM of glucose concentration (The linear regression equation is $y = 0.119x + 4.557$ and the linear regression coefficient is $R^2 = 0.750$).

In both cases, increase in current and glucose concentration have been observed within a range of 0 mM to 50 mM concentration. Steady state response was achieved within ca. 10 s. for each spike of glucose. During successive addition of 1mM of glucose, the increase in response was well defined. The sensitivity of the biosensor in acetate and phosphate buffer is found to be 0.22 µA/mM and 0.41 µA/mM respectively. The detection is sufficient for medical diagnostics purpose since the normal clinical range for glucose in blood is between 3.5 to 6.1 mM whereas abnormal glucose levels can reach as high as 20 mM [25].

F. Stability and Lifetime of the P (Py)/P (NMP)/pTS/GOx Electrode

The long term stability of the P(Py)/P(NMP)/pTS/GOx sensor electrode, in both acetate and phosphate buffer, was tested for 50 days. A steep decrease in current response at initial phase was observed in both cases. The decrease was more pronounced in acetate buffer. At later phases, the responses used to stabilize. A commendable stability of the sensor electrode of 47 days was recorded for Phosphate buffer where as for acetate the stability was about 32 days. The lifetime of the sensor was better than our earlier reported P (Py)/P (NMP)/$NaNO_3$/GOx sensor, an advantage purely attributed to the presence of pTS as dopant. The stability characteristics are given in Fig 6.

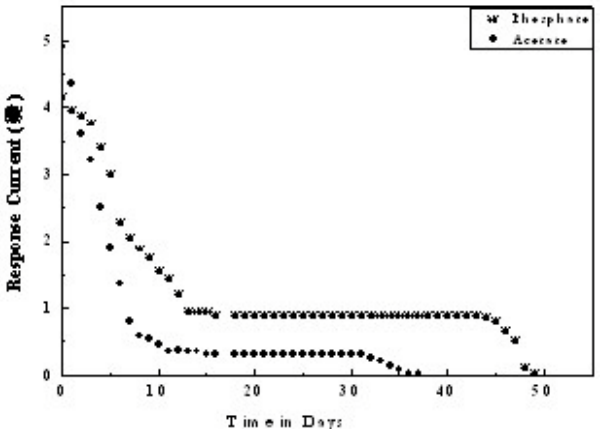

Fig.6 The stability of synthesized P(Py)/P(NMP)/pTS /GOx electrode in phosphate and acetate buffer (7.4 pH) for 5 mM of glucose concentration

IV. CONCLUSIONS

A P (Py)/P (NMP)/pTS composite film has been successfully synthesized by electrochemical technique. The process parameters were suitably optimized to support enzymatic activities for biosensing applications. Glucose Oxidase was immobilized by cross linking (via glutaraldehyde) on the composite matrix for fabrication of a glucose sensor electrode. The sensor exhibited an excellent linearity and fast sensing response to glucose concentration of 0-50mM which is a highly dynamic range suitable for medical diagnostics. A comparative study showed that the sensor operates better in phosphate buffer atmosphere than in acetate buffer. The

composite structure of P (py)/P (NMP) with pTS as a dopant showed an excellent durability of the sensor.

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